

WHAT IS CLAIMED IS:

1. A method of treating a neurological disorder associated with synaptic vesicle function, endocrinopathy or hormonal diseases, comprising administering a compound or agent that modulates a function or activity of an SV2 protein.
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2. A method of claim 1, wherein the neurological disorder is selected from the group consisting of seizure, epilepsy, Parkinson's disease, Parkinson's dyskinesias, migraine, Alzheimer's disease, neuropathic pain, essential tremor, cognitive disorders, and movement disorders.
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3. A method of claim 1, wherein the compound or agent binds to the levetiracetam binding site of an SV2 protein.
- 15 4. A method of modulating at least one function or activity of a SV2 protein in a cell, comprising exposing the cell to a compound or agent that binds to the levetiracetam binding site of the SV2 protein.
5. A method of claim 4, wherein the compound or agent modulates the binding of
20 levetiracetam to the levetiracetam binding site.
6. A method of discovering or modeling an interaction between an SV2 protein and a compound or agent selected from the group consisting of: levetiracetam, an analog or derivative of levetiracetam, or a compound or agent which competes with levetiracetam or an
25 analog or derivative thereof for binding to the levetiracetam binding site comprising:
 - a) contacting the SV2 protein with the compound or agent; and
 - b) measuring and analyzing the interaction of the SV2 protein with the compound or agent.
- 30 7. A method of claim 6 where the analysis is by proteolytic treatment of the SV2 proteins to observe a differential effect of binding of a ligand on proteolytic degradation.

8. A method of claim 6, wherein the analysis is by 3-dimensional modeling or other purely computational techniques.

5 9. A method of claim 8, wherein the 3-dimensional modeling is via nuclear magnetic resonance spectroscopy or X-ray crystallography.

10. A method of claim 6, wherein the analysis is by binding studies.

10 11. A method of any one of claims 6 or 10, wherein the SV2 protein is purified from natural sources

12. A method of claim 11 where the SV2 protein is purified from heterologously expressed protein driven from a cloned nucleotide inserted in an expression vector, in a
15 eukaryotic or prokaryotic host.

13. A method of identifying a levetiracetam binding site within an SV2 protein comprising;

- 20 a) contacting a SV2 protein or fragment thereof with a compound or agent selected from the group consisting of levetiracetam, an analog or derivative of levetiracetam, or a compound or agent which competes with levetiracetam or an analog or derivative thereof for binding to the levetiracetam binding site; and
- 25 b) determining the binding of the compound or agent with the SV2 protein or fragment thereof.

14. A method of claim 13, wherein the SV2 protein or fragment thereof comprises at least one amino acid substitution, deletion or addition.

30 15. A method of claim 14, wherein the addition, deletion or substitution of amino acid residues removes at least one glycosylation sites.

16. A method of claim 15, wherein the removal of glycosylation sites is *via* site-directed mutagenesis.

5 17. A method of claim 13, wherein the SV2 protein is a fusion protein comprising at least one SV2 protein or fragment thereof and a fusion partner.

18. A method of claim 17, wherein the fusion partner is a fusion tag.

10 19. A method of claim 18, wherein the fusion tag is a poly-His tag or glutathione-S-transferase.

20. A method of assaying the interaction between SV2 protein and a second protein comprising;

- 15 a) expressing SV2 protein and the protein of interest in a cell;
b) exposing the cell to a compound or agent which binds to the levetiracetam binding site; and
c) determining the interaction between the SV2 protein and the protein of interest.

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21 A method of claim 20, wherein the second protein is selected from the group consisting of: a cell membrane protein, a vesicle membrane protein, a cytoplasmic protein, a cytoskeletal protein, and an intracellular matrix protein.

25 22. A method of claim 20, wherein the protein of interest is synaptotagmin.

23. A method of claim 20, wherein the protein of interest is a member of the SNARE complex.

30 24. A method of claim 23, wherein the member of the SNARE complex is synaptic vesicle associated VAMP/synaptobrevin, syntaxin, or SNAP-25.

25. A method of claim 20, wherein the SV2 protein lacks at least one glycosylation site.

26. A method of identifying a compound or agent that modulates a neurological disorder associated with synaptic function, endocrinopathy or hormonal disease comprising;

- a) exposing a SV2 protein to the compound or agent; and
- b) determining whether the compound or agent modulates an activity of the SV2 protein.

27. A method of any one of claim 3, 4, 5, 6, 20, or 26, wherein the compound or agent is levetiracetam or an analog or derivative thereof, or an anti-SV2 antibody or fragment thereof.

28. A method of any one of claims 13 or 27, wherein the compound or agent competes with levetiracetam or an analog or derivative thereof for binding to the levetiracetam binding site.

29. A method of any one of claims 13 or 27, wherein the compound or agent is an anti-SV2 antibody or fragment thereof.

30. A method of claim 29, wherein the anti-SV2 antibody or fragment thereof binds to the levetiracetam binding site of SV2 protein.

31. A method of claim 29, wherein the anti-SV2 antibody or fragment thereof is selected from the group consisting of a polyclonal antibody and a monoclonal antibody.

32. A method of claim 31, wherein the antibody fragment is selected from the group consisting of an Fab fragment, Fab' fragment, F(ab')₂ fragment and an scFv fragment.

33. A method of claim 31, wherein the monoclonal antibody is selected from the group consisting of a chimeric antibody, a humanized antibody, and a human antibody.

34. A method of identifying a cellular response to a compound or agent selected from the group consisting of levetiracetam, an analog or derivative of levetiracetam, or a compound or agent which competes with levetiracetam or an analog or derivative thereof for binding to the levetiracetam binding site comprising:

- a) exposing cells expressing an SV2 protein to the compound or agent; and
- b) analyzing a change in the expression of a nucleic acid or protein in the exposed cell.

35. A method of any one of claims 20 or 34, wherein the step of exposing the cell to a compound or agent which binds to the levetiracetam binding site is carried out under conditions with a divalent cation concentration selected from the group consisting of less than about 1 μM , between about 1 μM and about 1000 μM , and at least about 1000 μM .

36. An isolated nucleic acid molecule comprising the nucleic acid sequence of SEQ ID NO: 5 or the complement thereof.

37. An isolated polypeptide comprising an amino acid sequence encoded by the isolated nucleic acid molecule of claim 123.

38. An isolated polypeptide of claim 124, comprising the amino acid sequence of SEQ ID NO: 6.

39. A method of claim 26, wherein the step of determining whether the compound or agent modulates an activity of the SV2 protein is selected from the group consisting of

- a) measuring transport of at least one monovalent cation or divalent cation across a membrane;
- b) measuring SNARE complex formation;
- c) measuring Ca^{2+} channel formation or activity;
- d) measuring SV2 interaction with at least one other protein;

- e) measuring transport of at least one substrate across a membrane; and
- f) measuring synaptic vesicle fusion, exocytosis, or synaptic vesicle recycling.

40. A method of claim 39, wherein the monovalent cation is selected from the group
5 consisting of H^+ , Cl^- , Na^+ and K^+ .

41. A method of any one of claim 35 or 39, wherein the divalent cation is selected from the group consisting of Ca^{2+} , Zn^{2+} , Pb^{2+} , Mg^{2+} , Mn^{2+} , Fe^{2+} and Cu^{2+} .

10 42. A method of claim 41, wherein the at least one divalent cation is Ca^{2+} .

43. A method of claim 39, wherein the at least one other protein is synaptotagmin.

15 44. A method of claim 39, wherein the at least one other protein is laminin-1.

45. A method of claim 39 wherein the at least one substrate is selected from the group consisting of amines, acetylcholine, excitatory neurotransmitters, GABA, serotonin, glycine or other amino acids, sugars and organic ions.

20 46. A method of identifying a binding partner for a SV2 protein, comprising:
a) exposing a SV2 protein or fragment to a potential binding partner;
b) incubating the protein or fragment and potential binding partner with (2S)-2-[4-(3-azidophenyl)-2-oxopyrrolidin-1-yl]butanamide; and
c) determining if the binding of (2S)-2-[4-(3-azidophenyl)-2-oxopyrrolidin-1-yl]butanamide to the protein is inhibited by the potential binding partner,
25 thereby identifying binding partner for the protein.

47. A method of identifying a compound or agent useful for the treatment of a neurological or endocrinological disorder, comprising:

30 a) exposing a SV2 protein or fragment to the agent and levetiracetam or an analog or derivative thereof; and

b) determining if the binding of levetiracetam or an analog or derivative thereof to the protein is modulated by the agent, thereby identifying an agent useful for the treatment of a neurological disorder.

5 48. A method of claim 47, wherein the levetiracetam or an analog or derivative thereof is directly or indirectly labeled.

49. A method of claim 47, wherein the SV2 protein or fragment is incubated with the levetiracetam or an analog or derivative prior to the agent, after addition of the agent, or
10 concurrent with the agent.

50. A method of claim 47, wherein the SV2 protein or fragment is incubated with levetiracetam.

15 51. A method of claim 47, wherein the neurological disorder is selected from the group consisting of epilepsy; epileptogenesis; seizure disorders; convulsions; withdrawal seizures; neurological disorders; bipolar disorders; mania; depression; anxiety; migraine; neuralgia; trigeminal neuralgia; chronic pain conditions; neuropathic pain; anaesthesia-related hyperexcitability; cerebral ischemia; head trauma; myotonia; excitatory states provoked by
20 drug or alcohol abuse, dependence or withdrawal; stroke; myoclonus; essential tremor; tics; Tourette's syndrome; dyskinesia; spasticity; movement disorders; neonatal cerebral haemorrhage; amyotrophic lateral sclerosis; Parkinson's disease; Alzheimer's disease; a neurodegenerative disease; and dementia.

25 52. A pharmaceutical composition comprising a compound or agent as identified in the method of any one of claims 26 or 47 said compound being different from a compound as described in Fig. 15.

53. A method of treating a neurological or endocrinological disorder which comprises
30 administering to an individual in need of such treatment a compound or agent as identified in the method of any one of claims 26 or 47 said compound being different from a compound as

described in Fig. 15.

54. A method according to claim 53, wherein the neurological disorder is selected from the group consisting of epilepsy; epileptogenesis; seizure disorders; convulsions; withdrawal
5 seizures; neurological disorders; bipolar disorders; mania; depression; anxiety; migraine; neuralgia; trigeminal neuralgia; chronic pain conditions; neuropathic pain; anaesthesia-related hyperexcitability; cerebral ischemia; head trauma; myotonia; excitatory states provoked by drug or alcohol abuse, dependence or withdrawal; stroke; myoclonus; essential tremor; tics; Tourette's syndrome; dyskinesia; spasticity; movement disorders; neonatal cerebral
10 haemorrhage; amyotrophic lateral sclerosis; Parkinson's disease; Alzheimer's disease; a neurodegenerative disease; and dementia.

55. A method according to claim 53 wherein the endocrinological disorders is selected from the group consisting of endocrinopathies involving hypersecretion or hyposecretion of
15 at least one hormone; gigantism; dwarfism; adrenal-medulla-related diseases; hypoglycemia; and circulation shock.

56. A method of any one of claims 1, 20, 26, 34, or 47, wherein the SV2 protein is SV2A.

20 57. A method of claim 56, wherein the SV2A protein comprises SEQ ID NO: 2.

58. A method of claim any one of claims 13, 27, or 47, wherein the analog or derivative of levetiracetam is (2S)-2-[4-(3-azidophenyl)-2-oxopyrrolidin-1-yl]butanamide.

25 59. A method of claim 58, wherein the analog or derivative of levetiracetam is selected from the group consisting of N-alkylated 2-oxo-pyrrolidine derivatives, N-alkylated 2-oxo-piperidinyll derivatives, and N-alkylated 2-oxo-azepanyl derivatives.

60. A method of identifying an agent useful for the treatment of a neurological or
30 endocrinological disorder, comprising:

a) exposing a SV2 protein or fragment to the agent;

b) incubating the protein or fragment and agent with (2S)-2-[4-(3-azidophenyl)-2-oxopyrrolidin-1-yl]butanamide; and

c) determining if the binding of (2S)-2-[4-(3-azidophenyl)-2-oxopyrrolidin-1-yl]butanamide to the protein is inhibited by the agent, thereby identifying binding partners for the protein.

61. A method of discovering or modeling an interaction between an SV2 protein, or fragment or derivative thereof, and a compound or agent selected from the group consisting of: levetiracetam, an analog or derivative of levetiracetam, or a compound or agent which competes with levetiracetam or an analog or derivative thereof for binding to the levetiracetam binding site comprising:

a) creating a 3-dimensional model of the SV2 protein, or fragments thereof, via either biochemical, biophysical, purely computational techniques, or some combination of these; and

b) creating 3-dimensional model of one or a collection of potential ligands that might potentially bind the SV2 protein.

62. A method of claim 61, further comprising using purely computational techniques to dock the 3-dimensional model of SV2 proteins with the 3-dimensional models of potential ligands.

63. A method of discovering or modeling an interaction between an SV2 protein and a compound or agent selected from the group consisting of: levetiracetam, an analog or derivative of levetiracetam, or a compound or agent which competes with levetiracetam or an analog or derivative thereof for binding to the levetiracetam binding site comprising:

a) determining a biochemical, pharmacological, organismal, cellular or molecular effect of a potential CNS active molecule in a genetically wild-type animal or in molecules, cells or tissues derived from such animals; and

b) comparing the measured effect of that compound in an equivalent study in a system with an SV2 protein knocked out or knocked down.

64. A method of isolating a functionally active membrane associated SV2 protein complex comprising:

- a) solubilizing tissues comprising the SV2 protein with a detergent; and
- b) isolating the SV2 protein complex.

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65. A method of claim 64, wherein the method further comprises purifying the SV2 protein complex by immunoaffinity.

66. A method of claim 65, wherein the SV2 protein complex is further purified to obtain
10 the SV2 protein.

67. A method of claim 64, wherein the detergent is n-dodecyl- β -D-maltoside or derivatives or analogs thereof.

15 68. A method of claim 64, wherein the tissues are brain membranes.

69. A method of claim 64, further comprising identifying the molecule or molecules complexed to the SV2 protein.

20 70. A method of any one of claims 64 to 69, wherein the SV2 protein is SV2A protein, SV2B protein, or SV2C protein.

71. A purified SV2 protein complex obtained by the method of claim 64.

25 72. A purified SV2 protein complex of claim 71, wherein the SV2 protein is SV2A protein, SV2B protein, or SV2C protein.